# mRNA ABUNDANCE OF SELECTED GENES IN THE LOIN MUSCLE OF PIGS WITH DIVERGENT CARCASS AND MEAT QUALITY TRAITS

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**Abstract – It is well accepted that DNA polymorphisms modulating gene expression can affect phenotypic traits, including carcass and meat quality traits. Our objective was to look for differences in mRNA abundance of candidate genes in the *longissimus* muscle of pigs showing divergent phenotypic values for such traits. The mRNA abundance was measured in muscle samples from 115 purebred pigs (Duroc, Landrace and Yorkshire) showing divergent carcass and meat quality traits. For each studied trait, animals were divided in 2 groups showing extreme phenotypic mean values (High (H) and Low (L)). The mRNA abundance of studied genes in H and L groups was then compared. Among studied genes, 6 (*ADIPOR1*, *LEP*, *PPARGC1A*, *PRKAG3*, *TNC* and *FABP4*) presented differences in gene expression between H and L animals in at least 2 of the 3 studied breeds. Most of those genes have known or suspected roles in pig's carcass and meat quality traits, and DNA polymorphisms were previously identified in their gene sequences. It remains to be determined whether these polymorphisms affect gene expression and phenotypic values. These genes may become promising markers for the selection of different carcass and meat quality attributes.**

**Key Words –Gene expression, *longissimus muscle***

## I. INTRODUCTION

In the past, pig breeding programs mainly focused on producing leaner and fast growing pigs. However, consumer's demands for higher meat quality products are gradually shifting breeding programs towards the selection of different meat quality attributes such as colour, marbling and tenderness. Besides nutritional and environmental factors, the genetic background of an animal also influences many meat quality parameters [1]. Some genes and markers have been included in pig selection programs to get rid of defects observed in meat quality (ex. Halothane, RN) or for improving production efficiency and product quality (ex. IGF2, MC4R). Since genetic components for carcass and meat quality traits are complex and controlled by multiple genes, there is a huge potential for identifying new genes or markers that are associated with these traits. The demonstration that DNA polymorphisms affecting gene expression greatly contributes to the phenotypic diversity of a population [2] further suggests that measurement of candidate gene mRNA abundance may provide new information to better understand the underlying mechanisms affecting carcass and meat quality attributes. Therefore, the objective of the current study was to look for differences in the mRNA abundance of some candidate genes in the *longissimus* muscle of pigs showing divergent phenotypic values for carcass and meat quality traits.

## II. MATERIALS AND METHODS

*Animals*- A total of 313 purebred pigs (from 16 breeding farms across Canada) entered the Deschambault test station (Quebec) in May 2010. Animals included Duroc (DD, n = 96), Landrace (LL, n = 101) and Yorkshire (YY, n = 116) breeds (2 castrates/litter). Pigs were fed *ad libitum* with cube-texture feed, following a 3-phase feeding program. Various measurements were collected on each pig during the test (Table 1). Pigs were slaughtered at a targeted live weight of 120 kg. Carcasses were tracked individually at the plant to allow *longissimus* muscle sampling immediately after slaughter and carcass and meat quality measurements 24 h *post mortem*.

*Gene expression*- To assess the mRNA abundance of studied genes in pigs having divergent

Table 1 Carcass and meat quality traits collected

| Measurements | Description |
|---|---|
| **Carcass traits** | |
| Carcass yield (kg/kg) | Hot carcass weight/Off test weight |
| Loin yield (%) | (Loin weight/Cold half carcass weight from primal cuts) x 100 |
| Backfat thickness (mm) | Off-test backfat measured ultrasonically |
| *Longissimus* muscle depth (mm) | Off-test loin muscle depth measured ultrasonically |
| Lean yield (%) | Carcass lean yield from a prediction equation based on backfat and muscle depth |
| Loin eye area ($cm^2$) | Picture + Image J software |
| ***Longissimus* meat quality traits** | |
| pH 24 h | Average of 2 pH measures |
| Colour score | Japanese scale colour (1 to 6) |
| Colour L* | Lightness/Minolta CR-300 |
| Colour a* | Redness/Minolta CR-300 |
| Colour b* | Yellowness/Minolta CR-300 |
| Drip loss (%) | EZ-DripLoss funnels |
| Shear force (N) | Texturometer |
| Marbling score | NPPC 2000 [3] |
| Intramuscular fat (% of wet sample) | Chemical measurement |
| Glycolytic potential (µmol of lactate equivalent/g tissue) | 2([glycogen] + [glucose] + [glucose 6 phosphate]) + [lactate] |
| Lactate dehydrogenase (LDH, IU/g tissue) | Glycolytic metabolism indicator |
| Citrate synthase (CS, IU/g tissue) | Oxidative metabolism indicator |
| Cooking loss (%) | 68°C [4] |

phenotypes for different measured traits, a total of 115 pigs were selected from the whole population. Pigs were selected based on an index allowing for the identification of top, middle and bottom groups for 5 selected traits (e.g. 24 h pH, colour L*, drip loss, shear force and intramuscular fat). Total RNA was extracted from muscle samples and cDNA synthesized as previously reported [5]. Quantitative real-time PCR analyses were performed using an Applied Biosystems 7500 Fast Real-Time PCR System (PE Applied Biosystems, Foster City, CA, USA) according to Farmer *et al.* [5]. The relative mRNA abundance of studied genes was determined using the standard curve method [6] and relative quantification values were normalized using reference genes (*ACTB, HPRT1* and *GAPDH*). Studied genes were selected based on their known or suspected roles in pig's carcass and meat quality traits. Genes were: adiponectin receptors 1 (*ADIPOR1*) and 2 (*ADIPOR2*), calpastatin (*CAST*), fatty acid binding protein 3 (*FABP3*) and 4 (*FABP4*), high mobility group AT-hook 1 (*HMGA1*), leptin (*LEP*), peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (*PPARGC1A*), protein kinase, AMP-activated, gamma 3 non-catalytic subunit (*PRKAG3*) and tenascin C (*TNC*).

*Statistical Analyses*- For each studied trait (Table 1) an automatic classification was first performed using the PROC FASTCLUS procedure of SAS. Within each breed, this allowed the formation of 2 groups showing extreme mean values (High (H) and Low (L)) and similar variances. Because extreme animals (e.g. H and L) in one studied trait are not necessarily the same for another trait, the number of pigs within each group may vary. A Student's t-test (PROC MIXED) was then performed on normalized quantity units to determine whether the mRNA abundance of studied genes is different between the 2 groups (H *vs* L). A Wilcoxon signed-ranked test was also used to confirm the Student's t-test.

## III. RESULTS AND DISCUSSION

Table 2 presents genes showing differences in gene expression between H and L animals in at least two of the three breeds under study for different carcass and meat quality traits. Among those, the *ADIPOR1* mRNA abundance was higher in LL ($P < 0.05$) and YY ($P \leq 0.10$) pigs having increased loin eye area, glycolytic potential and LDH activity. An increase in *ADIPOR1* mRNA abundance was also observed in pigs with low 24 h pH values ($P < 0.05$ for LL and YY) compared with high 24 h pH animals. Although a higher *ADIPOR1* mRNA abundance may seem beneficial with regards to the observed increase in loin eye area, this may not result in improved meat quality as an increased muscle growth potential is usually associated with a shift towards more glycolytic myofibres along with lower ultimate pH (as observed in the current study) and greater drip loss [7]. ADIPOR1 mediates adiponectin's action in several tissues including skeletal muscles [8]. A*DIPOR1* knockout mice become obese, glucose intolerant and present decreased energy expenditure, thus suggesting a key role in energy metabolism [9]. Ingelsson *et al.* [10] reported an

Table 2 mRNA abundance of studied genes in the *Longissimus* muscle of pigs with divergent carcass and meat quality traits.

| Phenotypes and genes | Duroc (n = 38) | | | | Landrace (n = 38) | | | | Yorkshire (n= 39) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H[a] | L[a] | SEM | *P* | H | L | SEM | *P* | H | L | SEM | *P* |
| *Loin* yield | | | | | | | | | | | | |
| *LEP* | 0.242 | 0.251 | 0.063 | 0.551 | 0.163 | 0.349 | 0.075 | *0.052* | 0.200 | 0.337 | 0.062 | **0.015** |
| Loin eye area | | | | | | | | | | | | |
| *ADIPOR1* | 0.627 | 0.605 | 0.028 | 0.605 | 0.530 | 0.445 | 0.027 | **0.025** | 0.612 | 0.546 | 0.035 | *0.097* |
| *Lean* yield | | | | | | | | | | | | |
| *PPARGC1A* | 0.226 | 0.116 | 0.034 | **0.016** | 0.226 | 0.245 | 0.045 | 0.821 | 0.351 | 0.164 | 0.067 | **0.026** |
| *Longissimus* colour score | | | | | | | | | | | | |
| *PRKAG3* | 0.508 | 0.443 | 0.040 | 0.239 | 0.252 | 0.398 | 0.037 | **0.002** | 0.504 | 0.637 | 0.048 | *0.067* |
| *TNC* | 0.260 | 0.126 | 0.048 | *0.105* | 0.148 | 0.112 | 0.052 | *0.093* | 0.188 | 0.062 | 0.036 | **0.022** |
| *Longissimus* pH 24 h | | | | | | | | | | | | |
| *ADIPOR1* | 0.649 | 0.610 | 0.033 | 0.260 | 0.426 | 0.515 | 0.031 | **0.038** | 0.480 | 0.638 | 0.038 | **0.006** |
| *Longissimus* marbling score | | | | | | | | | | | | |
| *FABP4* | 0.515 | 0.466 | 0.056 | 0.378 | 0.511 | 0.366 | 0.063 | **0.047** | 0.583 | 0.462 | 0.062 | *0.079* |
| *Longissimus* intramuscular fat (IMF) | | | | | | | | | | | | |
| *LEP* | 0.274 | 0.191 | 0.054 | 0.301 | 0.366 | 0.176 | 0.074 | *0.107* | 0.363 | 0.230 | 0.061 | *0.075* |
| Glycolytic potential | | | | | | | | | | | | |
| *ADIPOR1* | 0.612 | 0.631 | 0.026 | 0504 | 0.519 | 0.452 | 0.026 | **0.049** | 0.657 | 0.555 | 0.040 | *0.068* |
| *FABP4* | 0.423 | 0.560 | 0.048 | **0.044** | 0.372 | 0.451 | 0.052 | 0.653 | 0.418 | 0.546 | 0.059 | *0.064* |
| Lactate dehydrogenase (LDH) | | | | | | | | | | | | |
| *ADIPOR1* | 0.619 | 0.621 | 0.028 | 0.798 | 0.542 | 0.451 | 0.027 | **0.038** | 0.614 | 0.513 | 0.048 | *0.108* |
| Cooking loss | | | | | | | | | | | | |
| *TNC* | 0.099 | 0.289 | 0.049 | **0.005** | 0.130 | 0.108 | 0.037 | 0.623 | 0.098 | 0.151 | 0.037 | **0.049** |

[a] H, pigs showing the highest values for a specific trait; Low, pigs showing the lowest values for a specific trait.

inverse association between circulating adiponectin concentrations and the proportion of type IIb glycolytic muscle fibres. However, these authors did not study adiponectin receptors. In this study, higher *LEP* mRNA abundance was observed in LL and YY pigs having lower loin yield ($P \leq 0.05$) and higher IMF values ($P \leq 0.10$). These results are in accordance with previous studies showing positive correlations between plasma leptin and different body fat measurements in cattle, sheep and pigs [11]. The leptin roles in body composition and energy partition and the identification of several single nucleotide polymorphisms (SNPs) that associates with performance, carcass and meat quality traits [12], further suggest that *LEP* SNPs or gene expression may be used in breeding programs. The *PPARGC1A* gene is a coactivator that influences the expression of many genes, including peroxisome proliferator activated receptors (PPARs), known to have important roles in lipid metabolism and energy balance [13]. In this study, the *PPARGC1A* mRNA abundance was higher in DD and YY pigs having an increased lean yield ($P < 0.05$). Previous works have shown that *PPARGC1A* expression is higher in muscles containing more oxidative fibres and that it can enhance the number of oxidative muscle fibres [14]. Although the l*ongissimus* is considered as a glycolytic muscle, it will be interesting to investigate whether the observed increase in *PPARGC1A* gene expression is associated with an increase in oxidative fibres. The *TNC* mRNA abundance was higher in YY ($P < 0.05$) and in DD and LL ($P \leq 0.10$) pigs that had the highest values of colour score, whereas its mRNA abundance was lower in DD and YY pigs ($P < 0.05$) showing higher values of cooking loss. Interestingly, the *TNC* gene was shown to be differentially expressed in pork meat showing high/low drip loss and high/low pH [15]. This gene belongs to a family of genes involved in the coding of extracellular matrix (ECM) proteins, which are known to be major determinants in tissue water-holding capacity. In our study, a higher *TNC* mRNA abundance was observed in animals with higher 24 h pH values, but this was only observed in DD pigs. Moreover, there was no significant difference in drip loss values between H and L pigs (data not shown). The *FABP4* gene encodes for a fatty acid binding protein expressed in adipocytes [16]. Several polymorphisms were identified in the porcine *FABP4* gene, some of which are associated with IMF and backfat

thickness [17]. These results support our findings with regards to the loin marbling score as higher *FABP4* gene expression was observed in LL ($P < 0.05$) and YY ($P < 0.10$) pigs showing higher values of marbling score. Higher values of *FABP4* mRNA abundance were also observed in pigs having higher IMF but this was only true for LL ($P < 0.05$) pigs (data not shown). Further work is needed to understand the observed differences in *FABP4* gene expression in DD and YY pigs showing divergent glycolytic potential. The *PRKAG3* gene encodes for a muscle specific isoform of the regulatory γ subunit of AMPK, known to play a key role in energy homeostasis. Different DNA polymorphisms were observed in this gene with effects on glycolytic potential, pH and colour [18]. In the current study, *PRKAG3* mRNA levels were lower in LL ($P < 0.05$) and YY ($P < 0.10$) pigs having higher colour score values. For ultimate pH values, differences were observed in *PRKAG3* mRNA abundance between H and L pigs (e.g. H < L), but this was only true for YY pigs (data not shown).

## IV. CONCLUSION

In this study, differences in mRNA abundance of candidate genes are reported between H and L pigs showing divergent carcass and meat quality traits. Genotyping for several SNPs within studied genes was also conducted in our laboratory with the aim of identifying polymorphisms that impact gene expression. Combining genotyping and gene expression data will allow the identification of the most promising candidates that may become markers for the selection of different carcass and meat quality attributes.

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